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Detection of Single PSS Polymers on Rough Surface by Pulsed-Force-Mode Scanning Force Microscopy

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ABSTRACT

Samples of nanosize single polymers to be imaged by scanning force microscopy (SFM) are preferentially deposited on a molecularly flat substrate. However, many substrates are modified and acquire rough surfaces resulting in poor definition of single-polymer molecules. This work dealt with the imaging of single poly(sodium 4-styrenesulfonate) (PSS) polymers on a rough surface with a novel measuring mode, pulsed-force-mode (PFM). Adhesive forces can be mapped simultaneously with topographic imaging with this mode. Force–distance curves show the differences of adhesive forces of the tip/sample and the tip/surface couples, so that sample materials can be distinguished in terms of surface adhesive properties. PFM increases the roughness limit of substrates from 0.8 to 6.1 nm, allowing the observation of single molecules down to 1.0 nm height, and greatly expanding potential substrates for imaging of single polymers.

Scanning force microscopy (SFM) has been widely used to study the molecular structure and conformation of polymers.[1−5] In an earlier study, we have already demonstrated the application of tapping mode SFM for the imaging of polyelectrolytes, such as polyethylenimine (PEI) and PSS at the molecular level.[6−7] This mode is particularly reliable for the imaging of flexible long-chain polyeletrolytes without distortion of their native structures.[8] For the imaging of single polymers by tapping mode, the samples are usually deposited on an atomically or molecularly flat surface with an average roughness as small as a few angstroms (roughness < 0.5 nm/1μm^2).[9,10] Therefore, imaging is limited to adsorbed polymers on mica, graphite or self-assembled monolayers on which the small height of the polymers can be observed without interference from the background. However, other substrate surfaces may not be flat, or modified substrates used to study interaction or surface adsorption of polymers are often rough. When polymers are visualized on such surfaces having undulation close to the dimension of the polymers, it is quite difficult to differentiate the molecular particles from the material topography, even with the software background subtraction procedures. A procedure for correcting surface shape by modeling the surface with a low-order polynomial function and correcting the noise of scan line with a constant off-set line has been reported.[11] With this technique, the surface background correction due to orientation and noise may be improved up to 70% over commercial software. However, this technique does not work as well with rough surfaces, usually encountered in certain support materials, or irregular and random defects.[12] Another possible solution for imaging on rough surfaces is phase imaging SFM operating in tapping mode, which Holland et al. used to successfully distinguish individual fibrinogen protein molecules from the underlying surface,[13] while the roughness limit increased from 0.5 nm to 1.6−6 nm for uniform topography, the imaging appeared to be difficult when molecules spread extensively on the surface due to insufficient phase contrast. Because the spreading and collapsed conformation is the favorite model for charged polyelectrolytes adsorbed on reversibly charged surfaces, images of individual polymers on relatively rough surfaces have been difficult to obtain.

In this work, we attempted to exploit an alternative imaging mode, PFM, for the investigation of single polymers.[14−19] The topography of a sample may be measured simultaneously along with the mapping of surface properties, such as adhesion, surface-charge distribution, and viscoelastic behavior. This mode was also available for imaging with high scanning speed and high lateral resolution. Since no lateral force damage was observed and the normal force was precisely controlled, it was possible to image soft samples. We demonstrated the ability to visualize single PSS polymers on rough surfaces by adhesive imaging by PFM.

Rough surface substrates were prepared by first evaporating several nanometers of chromium as the adhesive layer on the silicon wafer followed by 200 nm of gold, and annealing at 150 °C for 2 h. As a control, a smooth surface sample, template-stripped gold with mica as template, was

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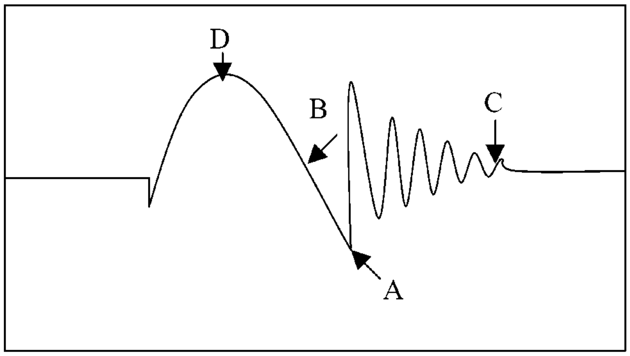

Figure 1. Scheme showing the force signal over a modulation period. Arrows indicate the points where the adhesion takes (A), starts (B), and stops trigger (C). Point D indicates the maximum force.

prepared.[9] Both the rough and smooth gold substrates were immersed into the adsorption solutions of 1.0 mM 12-aminododecanethiol for 2.5 h to form self-assembled monolayers. PSS polymers (molecular weight 70 000) were adsorbed onto the surfaces under diffusion control from dilute solutions.[7]

Single PSS molecules were imaged by SFM using a Nanoscope III SPM (DI Company, Santa Barbara) equipped with a PFM box (Witec Co., Germany). The force versus time curves, after sinusoidal *z* modulation, were recorded by an oscilloscope. Samples were imaged by PFM, with the collection of both topographic and adhesive data. The adhesive force was detected by comparing the difference between the zero and maximum negative forces at the jump-off point (point A in Figure 1) when the tip was released from the surface. While performing the measurement, point B was set as the adhesion start trigger and point C as the stop trigger. The most negative force signal between B and C was recorded as the adhesive signal. Measurements were carried out using silicon cantilevers with a spring constant of 3.0 N/m (Nanosensor, Germany) and a resonance frequency of 88 kHz. The cantilever was modulated at 700−800 Hz. Roughness analyses were performed according to the software provided by our SPM instrument.[20] The force−distance curves were obtained with the same SPM instruments equipped with a custom fluid cell. The normal spring constant *k* of the silicon cantilever (Ultrasharp, Silicon-MDT Ltd., Russia) used for the force measurements was determined using the method described by Sader et al.[21−22]

Topological details of the images revealed significant differences between the gold and template-stripped gold surfaces. The former appears dull and rough in contrast to the shiny smooth aspect of the latter one. The RMS roughness and undulation of the gold surfaces are indicated in Table 1. The lateral dimensions of the surface features are 800−1000 nm for all cases.

We measured the adhesive forces between a normal tip and multiple PSS polymer layer, either PSS polymer, multiple layers (tip/PSS), or an amino-terminated monolayer (tip/NH_3^+). The difference between the adhesive properties of the two couples, tip/PSS and tip/NH_3^+, was crucial for the following imaging based on adhesive force. The NH_3^+-modified gold substrate was immersed into a PSS polymer solution for 1 min to form a film of PSS (30−50 nm thick). The adhesive interaction was determined from the force versus displacement curves. The curves recorded with a NH_3^+ modified monolayer and a PSS-modified surface with the same Si_3N_4 tip are shown in Figure 2, and the corresponding histograms are shown in Figure 3. The pull-off forces correspond to adhesive interactions between the tip and the sample.[23−25] The tip/PSS interaction (1.8 ± 0.39 nN) was about twice as strong as the tip/NH_3^+(0.9 ± 0.25 nN) interaction. One would expect the negative charge of the Si_3N_4 tip to be repulsed from negatively charged PSS polymers and to be attached to the positively charged NH_3^+-terminated monolayer. Thus, the pull-off forces of tip/PSS should be smaller than tip/NH_3^+ in terms of electrostatic property. However, the larger tip/PSS pull-off force may be explained as the flexible quality of the PSS surface when the tip was brought into contact with a soft surface, such as PSS polymer, or that the sample may possibly be deformed, resulting in the real contact points impressing deeply down to A from B (as shown in Figure 4). This would make more PSS molecules adsorb onto the tip (points B and C) and expand the contact area of the tip and the polymers greatly, resulting in larger adhesive forces in terms of JKR theory.[23] Furthermore, when the tip is retracted from point C, the polymer chains could have been stretched, possibly resulting in the deformation of the chains under tension creating the high rupture force observed when the tip was further retracted (points D and E). The rupture force could be caused by the hydrogen bonds between the silanols and sulfonic groups, as well as van der Waals interactions.[26−29] Therefore, larger adhesive forces were observed when tips were forced to contact PSS polymer layers for larger contacting areas and high rupture forces.

Table 1: Topography of Surfaces

surface	RMS roughness (1 μm image)	undulation (nm)	surface feature size (nm)
template-stripped gold (200 nm)	0.077	0.8	800
gold (200 nm) coating on mica with annealing	1.1	6.1	1000

PSS polymers were first imaged with a regular tapping mode on rough surfaces (Figure 5). The RMS roughness of the substrate (Figure 5A) was 1.5 nm, with undulation of 6.1 nm. Additional spots were observed on the surface (Figure 5C) when compared to the gold substrate without PSS polymers. However, it was difficult to distinguish the PSS polymers from the original topography of underlying surface in height image by tapping mode.

The PSS polymers were observed by PFM−SFM on the same surfaces (Figure 5D). The topographic and adhesive data were collected simultaneously. The map of pull-off forces showed a difference between the adhesion of the NH_3^+ monolayer (Figure 5B) and the flexible PSS polymers (Figure 5D). Hence, the PSS polymers could be distinguished from

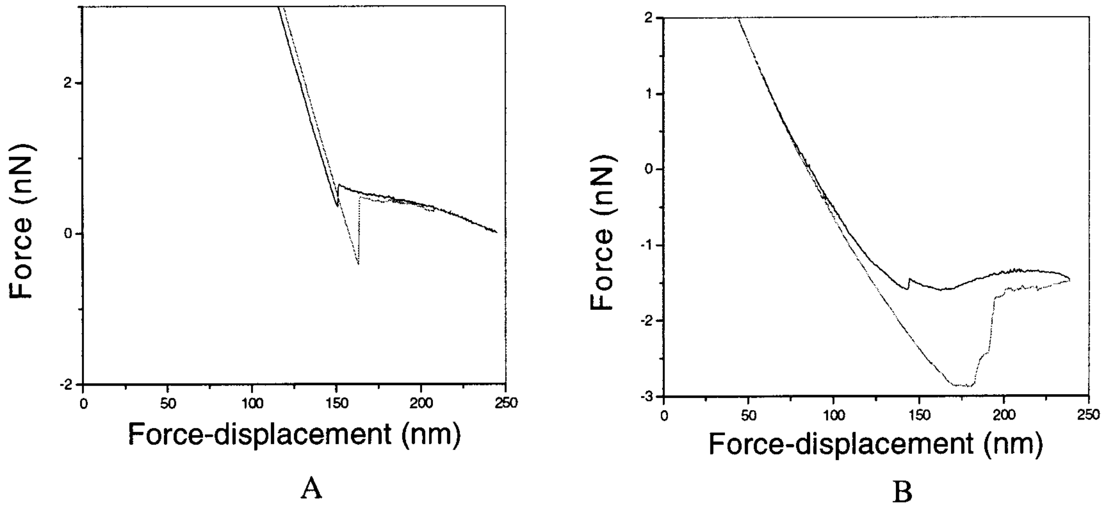

Figure 2. Typical force-displacement curves with a Si_3N_4 tip and a substrate modified with NH_3^+-terminated monolayer (A) and PSS polymers (B).

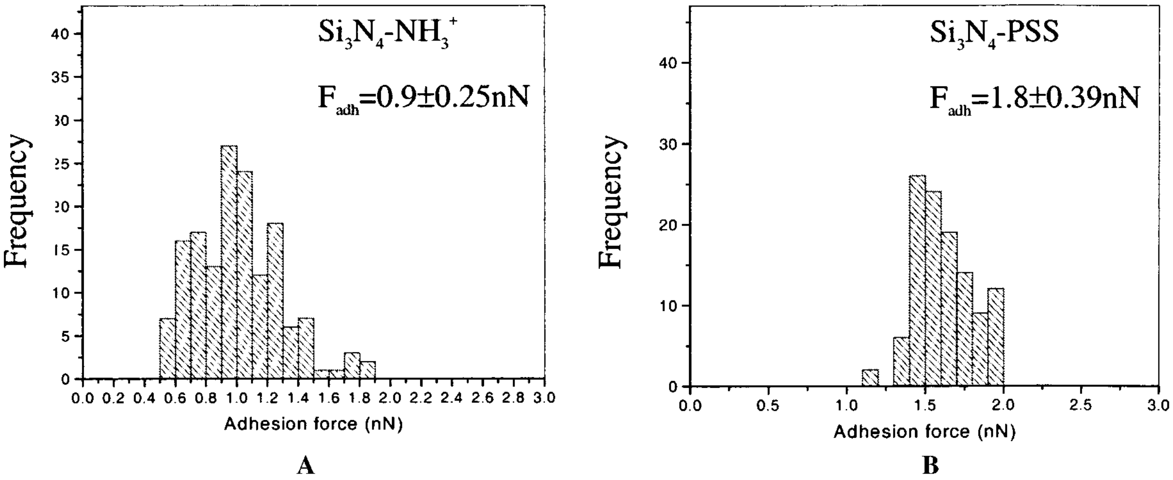

Figure 3. Histograms presenting the adhesion force distribution for a Si_3N_4-tip on NH_3^+ -functional SAM (A) and PSS polymer layer (B).

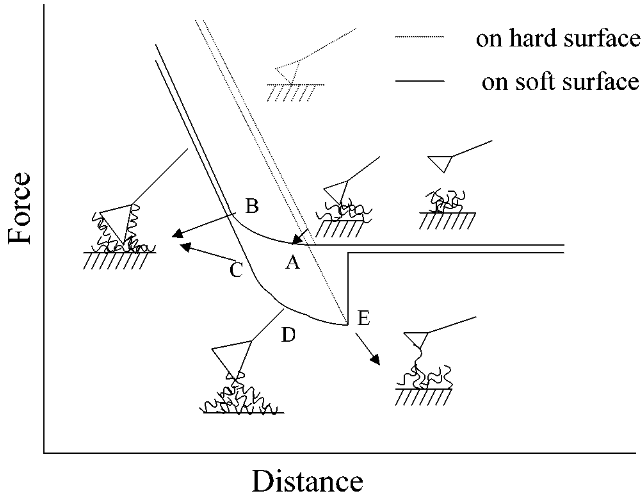

Figure 4. Typical force-displacement curves on a hard substrate or a soft thin sample and the schematic of approaching (A), touching (B), fully impressing (C), and separating (D) part of a force curve cycle.

the surface, a darker area with high adhesive forces corresponding to the PSS molecules indicating the raised area in the topography image. The adhesion was lower on the NH_3^+ monolayer modified gold (bright). Therefore, the adhesion signals in Figure 5 showed the corresponding structure of the PSS polymers on the surface.

In PFM, the maximum repulsive force was measured by using a sample-and-hold circuit, and was used for the feedback control for the tip. Therefore, it was possible to control the maximum repulsive force acting between the tip and the sample, which was especially important for the delicate samples.[14] The image quality in the adhesion image of PSS polymers was not poorer than images obtained on smooth surfaces. The reproducibility of the images suggested that the polymers adsorbed on the surface were stable under scanning by this mode.

In the previous work, we have demonstrated that PSS chains could be adsorbed dispersedly on positively charged surface from their dilution solution.[7] No aggregation of individual polymer molecules was observed in the condition, as shown in Figure 5. The single polymers of 27 nm width and 1.0 nm height on the flat substrate were identified. In terms of the large ratio of lateral and horizontal sizes, we concluded that the polymer molecules could be absorbed on

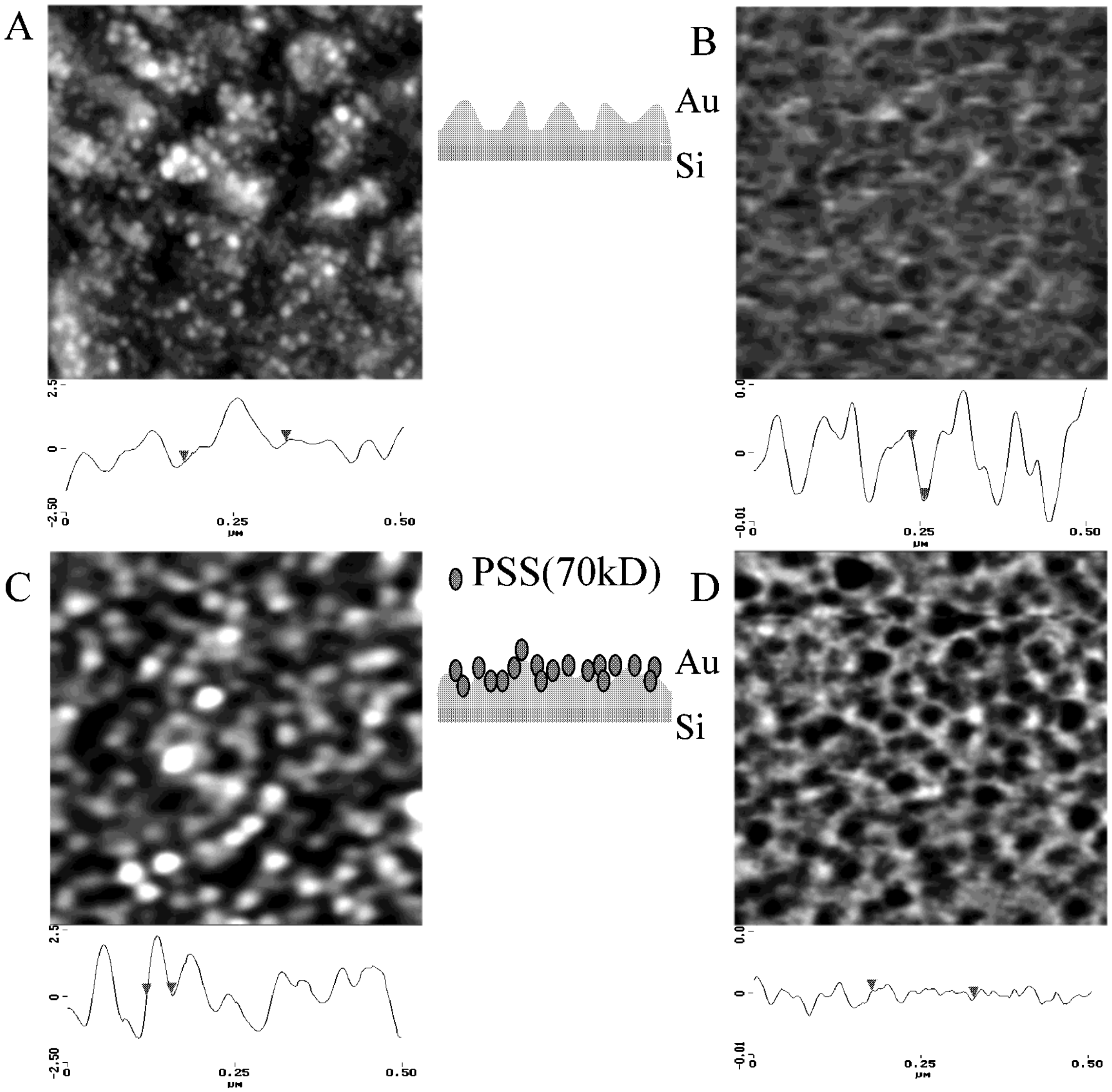

Figure 5. Tapping mode height images (A, C) and PFM adhesive images (B, D) of rough surface in the presence (C, D) of PSS polymers or absence (A, B). The scanning size is 500 nm × 500 nm for all cases. The height size is 5 nm for A, and 0.02 V for B.

the charged surface electrically with a collapsed conformation.[7] In this work, cross section analyses of over 50 spots in the images show the adhesive forces from 0.6 to 0.9 nN. The width range of PSS polymers was measured from 18 to 32 nm, with an average dimension of 27 nm. Our results were consistent with previously published data on smooth surface, therefore demonstrating the ability of the imaging single polymers with dimension of down to 1 nm height by PFM−SFM. Imaging individual PSS polymers on original gold surface without annealing (the undulation up to 10 nm) was not achieved in this study. The gold surface proved to be too rough to be distinguished from the adsorbed polymers.

In this work, single PSS polymers on rough surfaces, which were not observable in conventional topographic images, were detected successfully by PFM−SFM. The adhesive forces of the tip/PSS and tip/NH_3^+ couples were extrapolated from force−distance curves. PSS polymers could be distinguished from surface background in terms of adhesive property, with the molecular sizes being consistent with those on smooth surface. With this technique, the roughness limit for the observation of single polymers was increased from 0.8 to 6.1 nm. This allows the imaging of nanosize samples on the regular substrates. The technique is especially useful for the observation of soft samples, such as polyelectrolytes and biomacromolecules with larger adhesive forces against surface background, as well as samples with collapsed conformation and strong interaction with the underlying surface.

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References

(1) Sheiko, S. S. *Adv. Polym. Sci.* **2000**, *151*, 61−174.
(2) Pfau, A.; Schrepp, W.; Horn, D. *Langmuir* **1999**, *15*, 3219−3225.
(3) Regenbrecht, M.; Akari, S.; Forster, S.; Netz, R. R.; Möhwald, H. *Nanotechnology* **1999**, *10*, 434−439.
(4) Regenbrecht, M.; Akari, S.; Forster, S.; Möhwald, H. *J. Phys. Chem. B* **1999**, *103*, 6669−6675.
(5) Regenbrecht, M.; Akari, S.; Forster, S.; Möhwald, H. *Surf. Interface Anal.* **1999**, *27*, 418−421.

(6) Akari, S.; Schrepp, W.; Horn, D. *Langmuir* **1996**, *12*, 857−860.
(7) Zhu, M.; Schneider, M.; Papastavrou, G.; Akari, S.; Möhwald H. *Langmuir*, in press.
(8) Zhang, Q.; Ihhiss, D.; Kjoller, K.; Elings, V. B. *Surf. Sci*. **1993**, *290* (1−2), L688−L692.
(9) Hegner, M.; Wagner, P.; Semenza, G. *Surf. Sci*. **1993**, *291*, 39−46.
(10) Simpson, G. J.; Sedin, D. L.; Rowlen, K. L. *Langnuir* **1999**, *15*, 1429−1434.
(11) Pascual Starink, J. P.; Jovin, T. M. *Surf. Sci*. **1996**, *359*, 291−305.
(12) Dongmo, S.; Vautrot, P.; Bonnet, N.; Troyon, M. *Appl. Phys. A* **1998**, *66*, S819−823.
(13) Holland, N. B.; Marchant, R. E. *J. Biomed. Mater. Res*. **2000**, *51* (3), 307−315.
(14) Rosa-Zeiser, A.; Weilandt, E.; Hild, S.; Marti, O. *Meas. Sci. Technol*. **1997**, *8*, 1333−1338.
(15) Krotil, H.; Stifter, T.; Waschipky, H.; Weishaupt, K.; Hild, S.; Marti, O. *Surf. Interface Anal*. **1999**, *27*, 336−340.
(16) Miyatani, T.; Okamoto, S.; Rosa, A.; Marti, O.; Fujihira, M. *Appl. Phys. A* **1998**, *A66*, S349−352.
(17) Marti, O.; Stifter, T.; Waschipky, H.; Quintus, M.; Hild, S. *Colloid Surf. A* **1999**, *154*, 65−73.
(18) Okabe, Y.; Akiba, U.; Fuhihira, M. *Appl. Surf. Sci*. **2000**, *157*, 398−404.
(19) Okabe, Y.; Furugori, M.; Tani, Y.; Akiba, U.; Fuhihira, M. *Ultramicroscopy* **2000**, *82*, 203−212.
(20) The root-mean-square (RMS) average of the surface roughness value was calculated as the standard deviation of all the height values. The *z*-range values represented the undulation of the surface.
(21) Sader, J. E.; Larson, I.; Mulvaney, P.; White, L. R. *Rev. Sci. Instrum*. **1995**, *66* (7), 3789−3798.
(22) The force−distance curves were processed with the software named “force analysis” developed in this group, and the histograms of the adhesion force were obtained from 150 curves.
(23) Frisbie, C. D.; Rozsnyai, L. F.; Noy, A.; Wrighton, M. S.; Lieber, C. M. *Science* **1994**, *265*, 2071−2074.
(24) Akari, S.; Horn, D.; Keller, H.; Schrepp, W. *Adv. Mater.* **1995**, *7*, 549−551.
(25) Papastavrou, G.; Akari, S. *Colloid Surf.* **2000**, *164*, 175−181.
(26) Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. *Science* **1997**, *275*, 1295−1297.
(27) Li, H. B.; Rief, M.; Oesterhelt, F.; Gaub, H. E. *Adv. Mater*. **1998**, *10*, 316−319.
(28) Li, H.; Liu, B.; Zhang, X.; Gao, C.; Shen, J.; Zou, G. *Langmuir* **1999**, *15*, 2120−2124.
(29) Grandbois, M.; Beyer, M.; Rief, M.; Clause-Schaumann, H.; Gaub, H. E. *Science* **1999**, *283*, 1727−1730.

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